

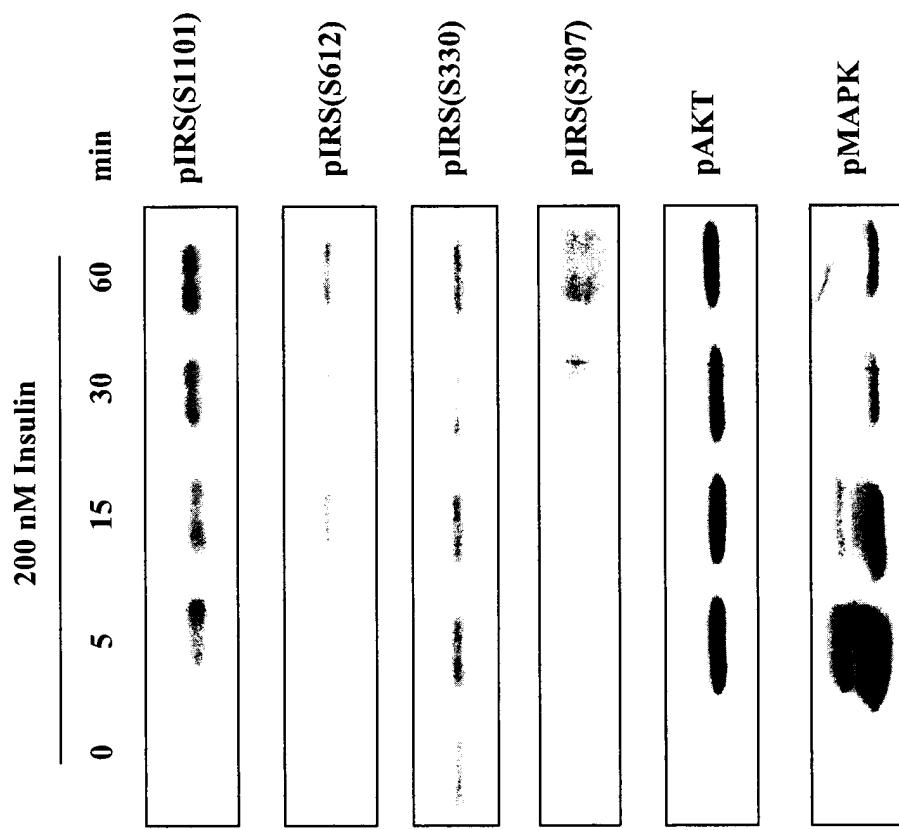
Figure 1: Human IRS-1 protein sequence (SEQ ID NO: 1)

1 masppesdgp sdvrkvgylr kpksmhkrrf vlaaseagg parleyyene kkwrhkssap  
61 krsiplescf ninkradskn khlvalytrd ehfaiaadse aeqdswyqal lqlhnarakgh  
5 121 hdgaaalgag ggggscsgss glgeagedls ygdvppgpaf kevwqvilkp klgqtknli  
181 giyrlcltsk tisfvklnse aaavvlqlmn irrcghsenf ffievgrsav tgpgefwmqv  
241 ddsvvaqnmh etileamram sdefrprsks qsssncsnpi svplrrhhln npppsqvglt  
301 rrsrtesita tspasmvggk pgsfrvrass dgegtmsrpa svdgspvsps tnrthahrhr  
361 gsarlhppln hsrsipmpas rcspatspv slsssstsgh gstsdcfpr rssasvsgsp  
10 421 sdggfissde ygsspcdftrs sfrsvtpdsi ghtppargee elsnyicmgg kgpstltapn  
481 ghyilsrggn ghrctpgtgi gtspalagde aasaadldnr frkrthsagt sptithqktp  
541 sqssvasiee ytemmpaypp gggsggrlpg hrhsafvptr sypeeglemh plerrgghhr  
601 pdsstlhtdd gympmspgva pvpngrkgsg dympmspksv sapqqiinpi rrhpqrwdpn  
661 gymmmmspsgg cspdiggps sssssnavp sgtsygklwt ngvgghhshv lphpkppves  
15 721 sggkllpctg dynmnmspvgd sntsspsdcy ygpedpqhkp vlsyyislprs fkhtqrpgep  
781 eegarhqhlr ldstssgrlly aataddssss tssdslgggy cgarlepslp hphhqvlqph  
841 lprkvdtaaq tnsrlarptr lslgdpkast lprareqqqq qqpllhpppep kspgeyvnie  
901 fgasdqsgyls gpvafhssps vrcpsqlqpa preeetgee ymkmdlgpgr raawqestgv  
961 emgrlgpapp gaasicrptr avpssrgdym tmqmscprqs yvdtspaapv syadmrtgia  
20 1021 aeevslprat maaassssaa sasptgpqga aelaahssll ggpqgpggms aftrvnlsrn  
1081 rnqsakvira dpqgcrrrhs **setfsstpsa** trvgntvpgf agaavggggg sssssedvkr  
1141 hssasfenvw lrpgelggap kepaklcgaa gglenglnyi dldlvkdfkq cpqectpepq  
1201 ppppppphpqp lgsgessstr rssedlsaya sisfqkqped rq

**Figure 2: Human IRS-2 protein sequence (SEQ ID NO: 2)**

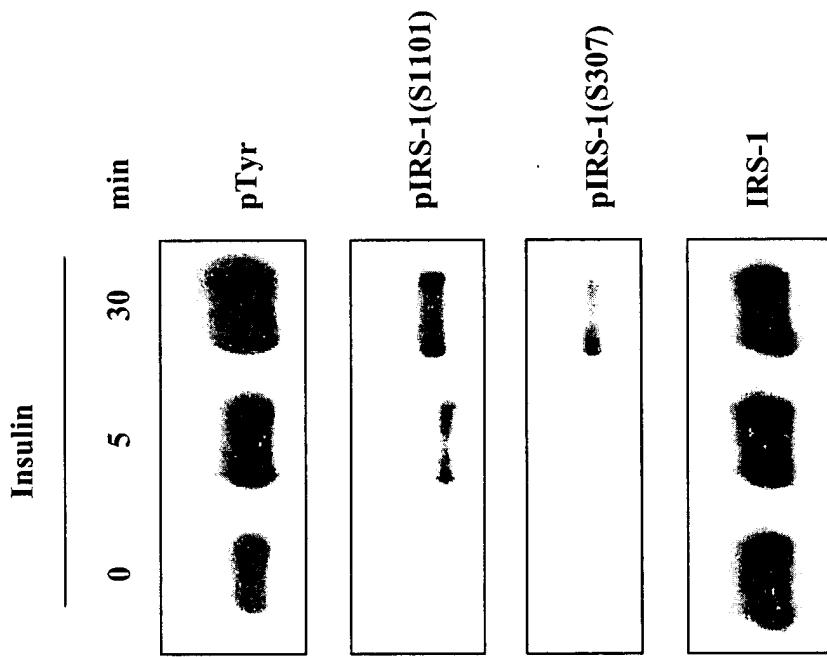
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61 tagggsapqp prleyyesek nwrskagapk rvialdccln inkradpkhk ylialytkde  
121 yfavaaaeneq eqegwyralt dlvsegrraaa gdappaapa ascsaslpga vggsgagaaga  
5 181 edsyglvapa taayrevwqv nlkpklgqs knltgvrylc lsartigfvk lnceqpsvtl  
241 qlmnirrcgh sdsfflievg rsavtgpel wmqaddsvva qnihetilea mkalkelfef  
301 rprsksqssg ssathpisvp garrhhhlnv lppsqtgtlvr rsrtdslaat ppaakcsscr  
361 vrtasegdgg aaagaaaaaga rpvsvagspl spgpvraps rshtliggcr aagtktwhcfc  
421 aggglqhsrs msmpvehlpp aatspgsrss ssdhgwgsyp pppgphpllp hplhhgpgqr  
10 481 pssgsasasg spsdpgfmsl deygsspgdl rafcshrsnt pesiaeetppa rdgggggefy  
541 gymtmdrpls hcgrsyrrvs gdaaqdldrg lrkrtylsit parqrpvlpq ssasldeytl  
601 mratfsgsag rlcpscpass pkvayhpype dygdieigsh rssssnlgad dgympmtpga  
661 alagsgsgsc rsddymppmsp asvsapkql qpraaaaaaaa avpfagpagp aptfaagrtf  
721 pasgggykas spaesspeds gymrmwcgsk lsmehadgkl lpngdylnvs psdavttgtp  
15 781 pdffsaalhp ggeplrvpg ccyslprsy kapytcggds dqyvlmsspvc grileeerle  
841 pqatpgptqa asafgagptq pphpvvpspv rpsgrpegf lgqrgravrp trlsleglps  
901 lpsmheyplp pepkspgeyi nidfgepgar lsppapplla saasssslls asspalslgs  
961 gtpgtssdsr qrsplsdymn ldfsspkspk pgapsghpvg sldgllspea sspypplppr  
1021 psaspssslq ppppppapge lyrllpasav ataqqpgaaas slssdtgdng dytemafgva  
20 1081 atppqpliaap pkpeaarvas ptsgvkrlsl meqvsgveaf lqasqppdpf rgakviradp  
1141 qggrrrhsse tfssttvtp vspsfahnpk rhnsasvenv slrksseggv gvgpgggdep  
1201 ptsprqlqpa pplapqgrpw tpgqpgglvg cpgsggspmr retsagfqng lkyiaidvre  
1261 epglppqppq pppplpqpgd ksswgrtrsl gglisavgvg strggcggpg pgapapcptt  
1321 yaqh

**Figure 3: Serine phosphorylation of IRS-1 in CHO/IR/IRS-1 cells**

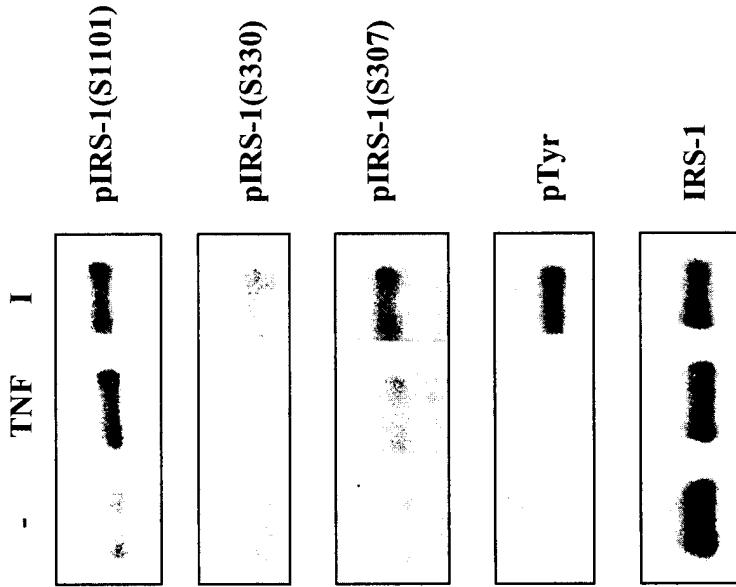


Sheet 4 of 14  
Antibodies Specific for Phosphorylated Insulin Receptor  
Substrate-1/2 (Ser1101/Ser1149) and uses thereof  
Polakiewicz et. al.  
Atty. Docket: CST-209

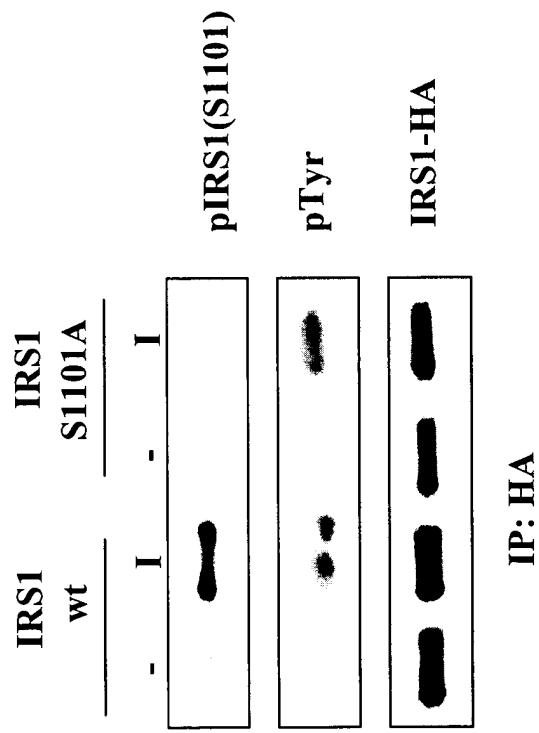
**Figure 4: Phosphorylation of IRS-1 in 3T3L1 fat cells**



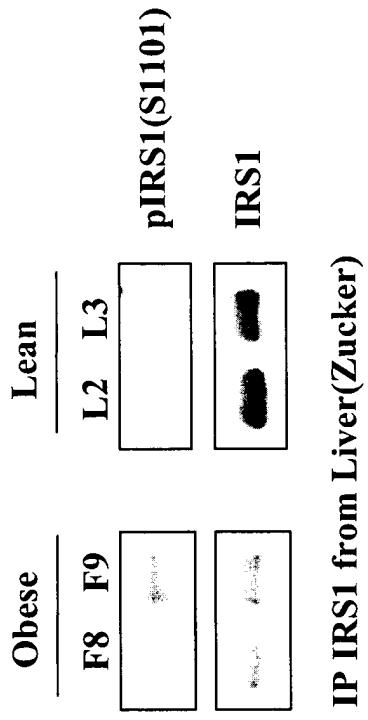
**Figure 5: Phosphorylation of IRS-1 in L6 myocytes**



**Figure 6: Specificity of pIRS-1(Ser1101) antibody**



**Figure 7: Phosphorylation of IRS-1 at Ser1101 in liver of Zucker rats**



**IP IRS1 from Liver(Zucker)**

**Figure 8: Phosphorylation of IRS-2 at Ser1149 in CHO cells**

**IRS-1 S1101: GCRRRHSS\*SETFFSSTP**

**IRS-2 S1149: GGRRRHSS\*SETFFSSTT**

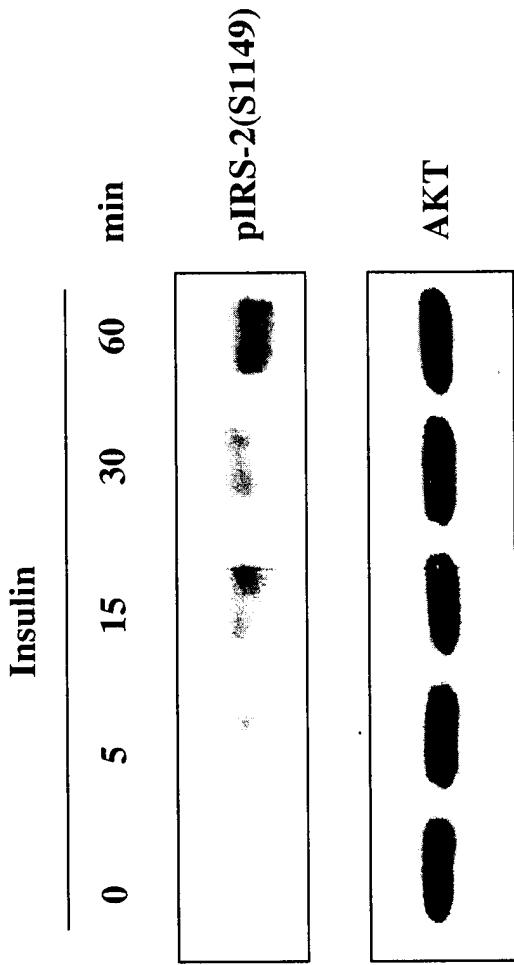
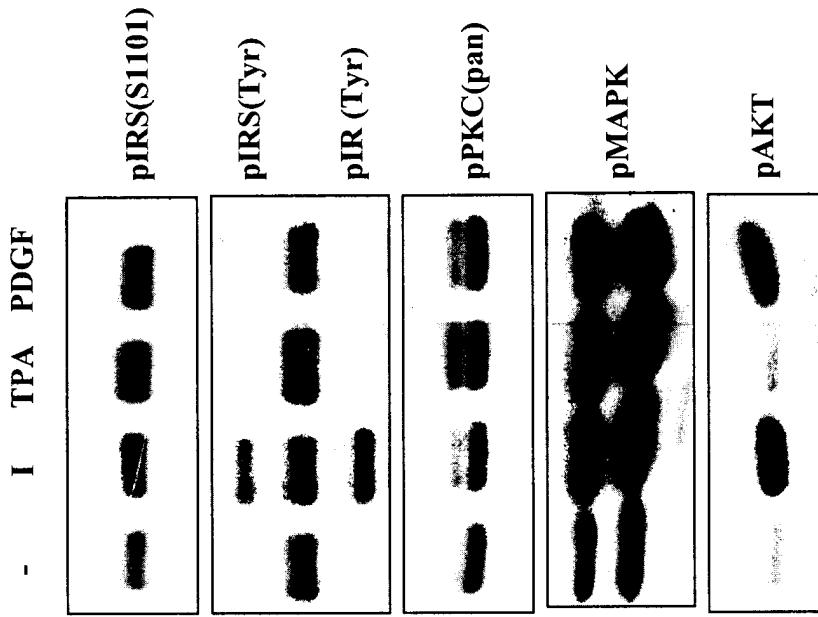
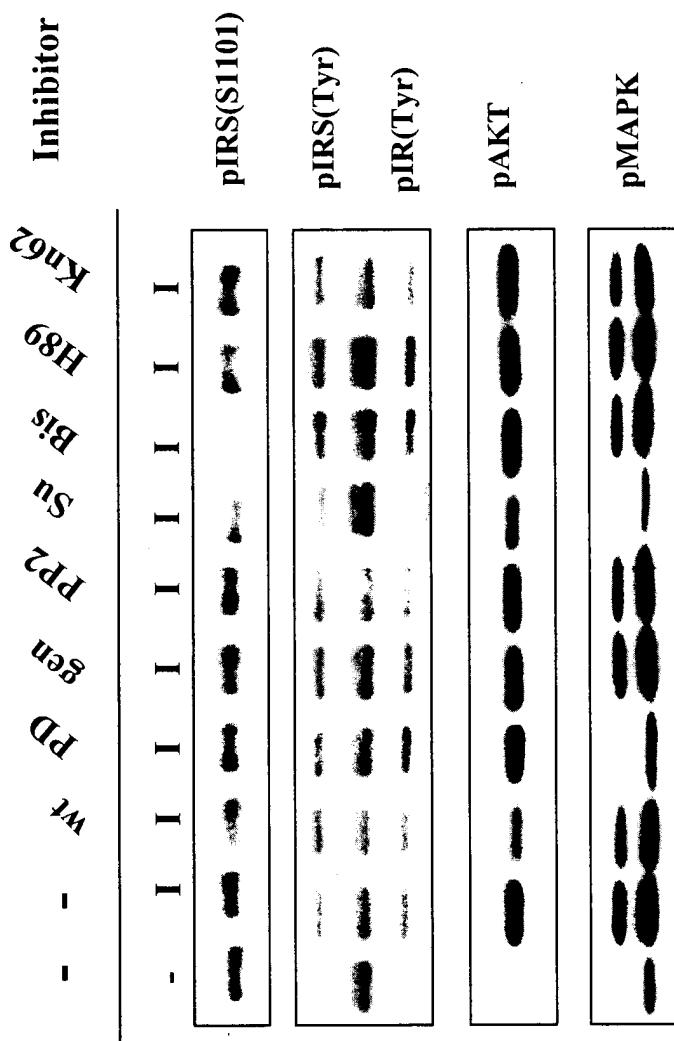


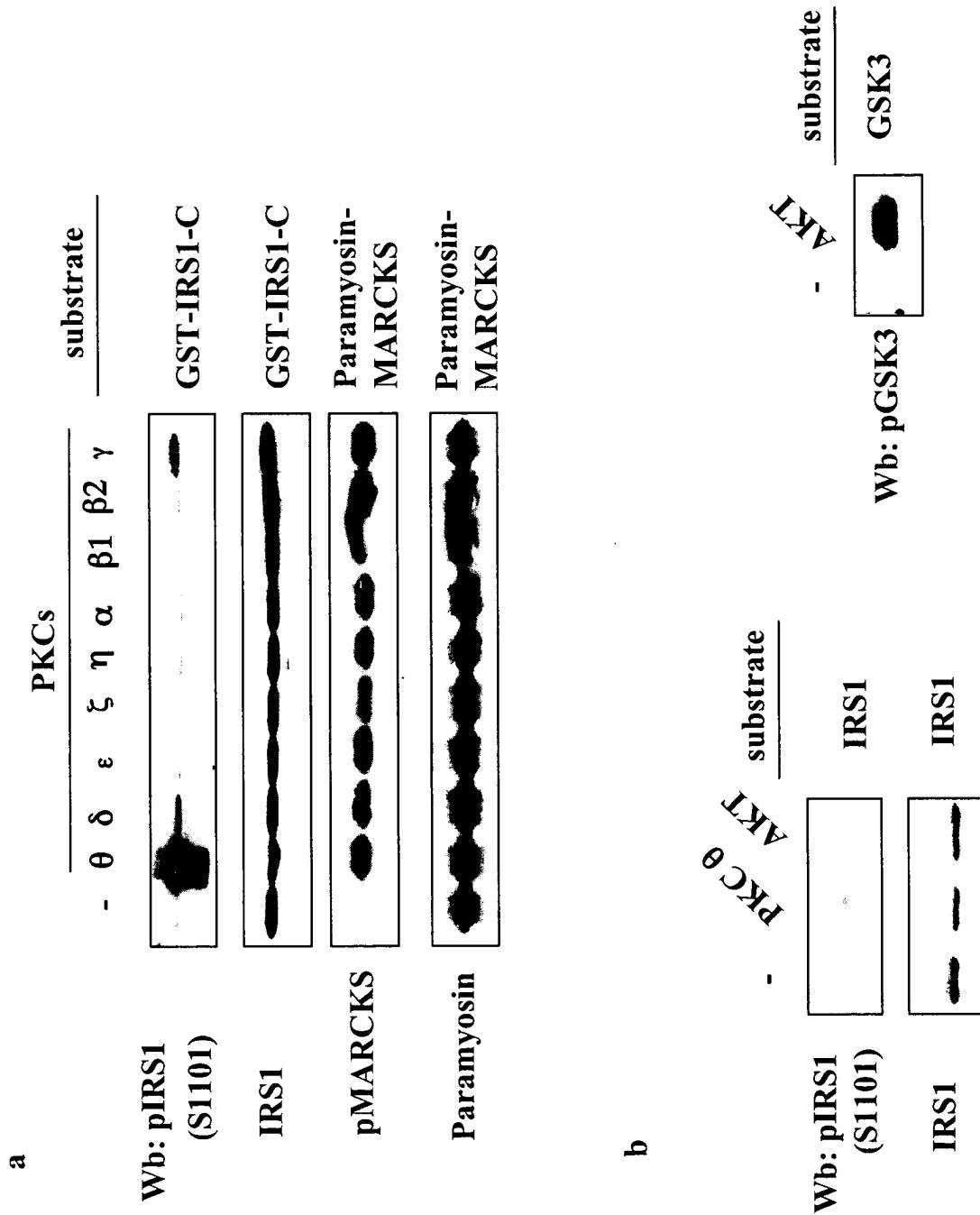
Figure 9: Phosphorylation of IRS1 at Ser1101 correlates with PKC activation



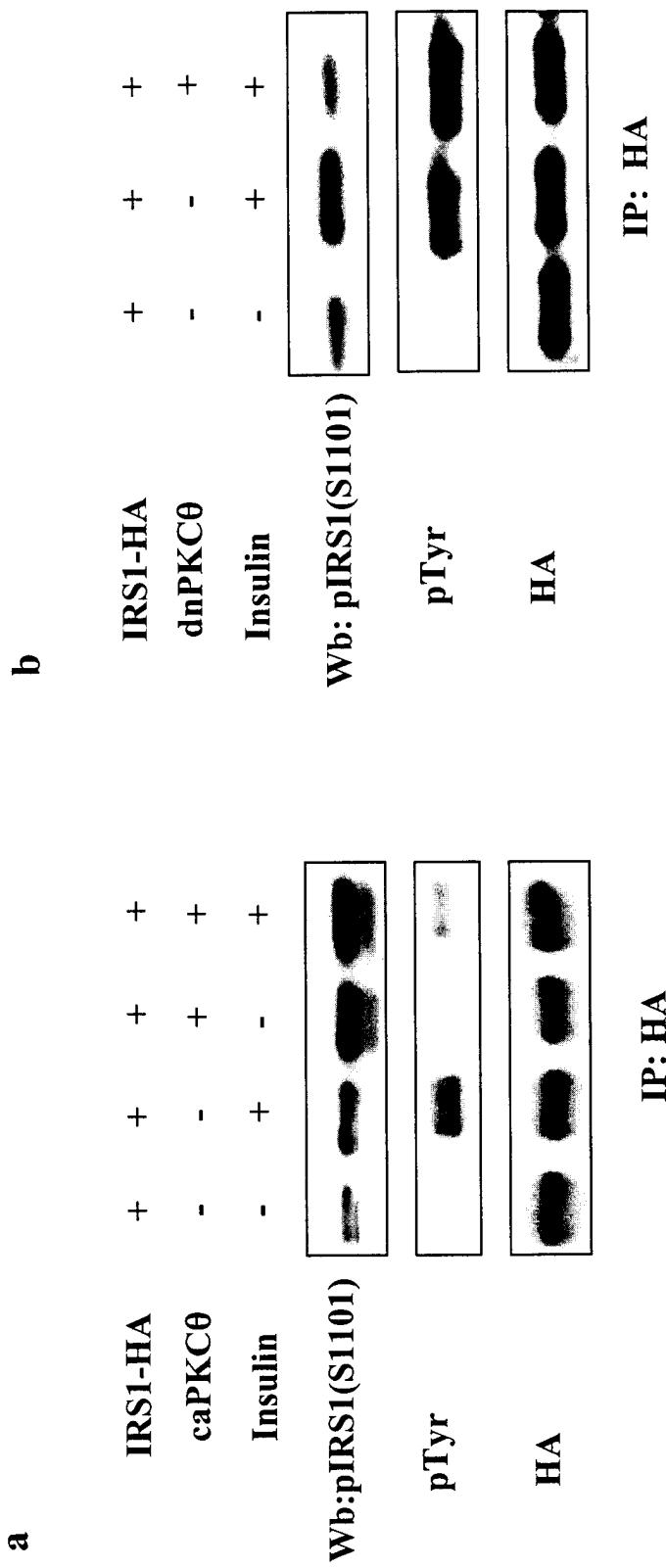
**Figure 10: Phosphorylation of IRS1 at Ser1101 is blocked by PKC inhibition**



**Figure 11: PKC $\theta$  kinase phosphorylates IRS-1(Ser1101) in vitro**



**Figure 12: PKC $\theta$  kinase phosphorylates IRS-1(Ser1101) in vivo**



**Figure 13: Mouse IRS-1 protein sequence (SEQ ID NO: 3)**

1 masppdt dgf sdvrkvgylr kpksmhkrff vlr aaseagg parleyyene kkwrhkssap  
61 krsiplescf ninkradskn khlv alvalytrd ehfaiaadse ae qds wyqal lql hnra ka h  
121 hdg agg g cgg scsg ssg vg e aged lsy dtg pg paf kevwq vil kpk glg q tkn ligiy r l  
181 cltsktisfv kln sea aavv lql mnirrcg hsen f fiev grs avt gpge fw mqv dds v  
241 aqnm hetile amramsdefr prsksq sss cs npis vpl r hh lnn pps qvg ltrrs rt  
301 esit atspas mv ggkpgsfr vrass dge gt msrp asvdgs p vsp stnr th ahr hrg ssrl  
361 hp pln hsrsi pm pssrcs ps at spv slss st sgh g stsd cl fpr r ssas vsg sps dgg f  
421 issdey gssp cd frssfr sv tp dsl ght pp argee el sny ic m ggk g a st laap n ghy il  
481 sr gg n ghry i p g anlg t spa l pg dea a gaa dld nfr krt hs agt sptis hq ktp s qss v  
541 asie ey temm paay ppg ggs ggr lpg y rh s af vpth s type egle mh hler rg gh hr pdts  
601 nl ht dd gymp ms pg vav vp s nr kg ng dymp ms pk svs apq q iin pir rhp qr vd p ngy mm  
661 ms psg scs pd iggg sss sss ssa aps gssy gkp wtng vgg hh th al phak pp v esgg gkl  
721 lp ct gd ym nm sp vg ds nt ss p se cy yg ped pq hk p vls yy sl prs fk ht q rp ge pe egar  
781 hq h l ls ss s g rl ry tatae dss sst ss ds lgg gyc gar p ess lth phhh vl qph lpr kv  
841 dt aaqtnsrl ar p trl s lg d pk ast lpr vr eq qqq qqssl h p e p ksp ge yvn ie fg sg q  
901 pg yl agp ats rss psv rc pp ql hpa preet gse ey mn md l g p grr at wq e sgg vel grig  
961 papp g sat vc rptr sv pn sr gd ym tm qig c pr qsy vdt sp v apv sy admr tg ia aek as l  
1021 pr pt ga a app p sst ass sv tp qgata e qath ss ll gg p qgp gg ms a ftr vn lsp nhn q s ak  
1081 vir adt tag cr rr hs set fsa p tra gnt vpf gaga a vgg sg ggg ggg sed v kr hss as fe n  
1141 vw lr p g d lgg vs kes a pvc g aaggleksln yidl laker sq dc psq qqs lpp pp hq pl  
1201 gs ne gn s p rr ss ed ls ny as is f qk qped r q

**Figure 14: Mouse IRS-2 protein sequence (SEQ ID NO: 4)**

1 masaplgppp asgggdgpnl nnnnnnnnhs vrkcgylrkq khghkrffvl rgpgtggdea  
61 saaggsppqp prleyyesek kwrskagapk rvialdccln inkradakhk ylialytkde  
121 yfavaaeneq eqegwyralt dlvsegrsge ggsgttggsc saslpvgvlgg sagaagcddn  
181 yglvtptav yrevwqvnlk pkglgqsknl tgvyrlcsa rtigfvklnc egpsvtlqln  
241 nirrcghsds fffievgrsa vtgpgelwmq addsvvaqni hetileamka lkelfefrpr  
301 sksqssgssa thpisvpgar rhhhlnvnlp sqtgtvrrsr tdslaatppa akctscrvt  
361 asegdggaaag gagtaggrpm svagsplspg pvrapsrsh tisagcggrp skvtlapagg  
421 alqhsrsnsm pvahtsppaat spgslssssg hgsgsyplpp gshphlphpl hhpqgqrpss  
481 gsasasgsps dpgfmsldey gsspgdlraf sshrsntpes iaetppardg sggelygym  
541 mdrplshcgr pyrrvsgdga qdldrglkrk tyslttparq rqvpqpssas ldeytlmrat  
601 fsgssgrlcsp sfpasppkva ynpypedygd ieigshksss snlgaddgym pmtpgaalrs  
661 ggpnscsksdd ympmsptsvs apkqilqprl aaalppsgaa vpappsgvgr tfpvnggyk  
721 asspaesspe dsgymrmwcg sklsmenpdp kllpngdyln kspseagttag tppdfsaalr  
781 ggseglkgip ghcysslprs ykapcscsgd ndqyvilmssp vgrileerl epqatpgagt  
841 fgaaggshfq phhsavpssm rpsaiggrpe gflgqrcrav rptrlslegl qtipsmqeyp  
901 lptepkspge yinidpgeag trlsppappl lasaassssl lsasspassl gsgtptgssd  
961 srqrspisdy mnldpssspks pkpstrsgdt vgsmdgllsp easspypplp prpstspssi  
1021 qqplppapgd lyrlippasaa tsqqptagss mssepgdngd ysemafgvaa tppqpvivapp  
1081 kpegarvasp tsglkrisl dqvsgveafl qvsqppdphr gakviradpq **ggrrrhssset**  
1141 **fsstttvtpv** spsfahnskr hnsasvenvs lrkssegsst lgggdeppts pgqaqplvav  
1201 ppvpqarpwn pgqpgaligc pggssspmrr etsvgfqngl nyiaidvrge qgslaqsqeq  
1261 pgdknswsrt rslggllgtv ggsgasgvvcg gpgtgalpsa styasidfls hhlkeatvvk  
1321 e